Gamma Irradiation Effects on Thiamin and Riboflavin in Beef, Lamb, Pork, and Turkey

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ABSTRACT

A study was made of the loss of thiamin and riboflavin due to gamma irradiation of beef, lamb and pork *longissimus dorsi*, turkey breast and leg muscles. Thiamin losses averaged 11%/kiloGray (kGy) and riboflavin losses 2.5%/kGy above three kGy. The rate of loss of thiamin in beef was higher than that in lamb, pork and turkey leg, but not turkey breast, with losses of 16%/kGy in beef and 8%/kGy in lamb. The rate of thiamin loss was not related to sulfhydryl, protein, moisture, fat or water content, pH or reducing capacity by redox titration. Loss of riboflavin was not different among species. Any detriment from such slight losses would seem to be more than compensated by the advantage of controlling bacteriological contamination by irradiation processing.

Key Words: meats, gamma irradiation, poultry, thiamin, riboflavin

INTRODUCTION

THE USE OF GAMMA IRRADIATION at doses of 0.3 to 1.0 kGy has been approved for elimination of trichina in pork (CFR, 1993a) and of 1.5 to 3.0 kGy for pasteurization of chicken (CFR, 1993b). With regard to outbreaks of *E. coli* 0157:H7 in ground beef, food irradiation could be a partial solution for ensuring the safety of hamburgers (FCN, 1993). Use of low-dose irradiation has been proposed for general approval for all meats (Merritt and Taub, 1983). The basis for the proposal is the concept of "chemiclearance" which states that the effects of irradiation are primarily based on the received dose. It also indicates it is possible to extrapolate results from higher to lower doses, and that the irradiation of similar substrates would have comparable results. We have previously reported the effects of gamma radiation on the loss of vitamins B_1, B_2, B_6, B_{12} and niacin in pork chops and chicken breasts (Fox et al., 1989) and the loss of thiamin in ground beef, chicken and pork (Fox et al., 1993). Our current objective was to test the hypothesis that the effect of low-dose gamma radiation on thiamin and riboflavin would be the same in muscle tissues regardless of species. We tested the effects of gamma radiation on beef, lamb, pork and turkey under identical conditions. For turkey, both breast and leg muscles were studied. The breast consists primarily of white muscle fibers and leg predominantly of red fibers thus the two have differing physiological properties (Cassens and Cooper, 1971). In addition to thiamin and riboflavin, we studied other muscle tissue components, specifically free sulfhydryl groups, pH and total reducing capacity.

MATERIALS & METHODS

Meats

All meats were purchased locally on the day after slaughter. Pork was purchased from Leidy of Soudertown, PA, beef (steer) from Carl Venezia of Conshohocken, PA, lamb and turkey from C. Fehl's of Spring House, PA. The meats were the *longissimus dorsi* of the mammals, and the breast and all of the leg muscles of the turkey. An attempt was made to keep the number of individual animals to a minimum but it was not always possible. The beef came from at least three animals, the pork from three, the lamb from six or more and the turkey from ten. All muscles were carefully trimmed of fat, then diced into cubes and frozen on dry ice. The meat was then pulverized in a Hobart silent cutter to yield a homogeneous powder which formed a smooth paste when thawed, suitable for precisely determining changes in muscle components. This preparation has been compared with ground and whole muscle samples and, as far as vitamin loss is concerned, yielded the same results, only with greater precision. The powder was allowed to thaw, packaged into Cryovac E-300 poultry bags (oxygen transmission 4000 $cm^3/m^2/24$ hr, 1 atm at 22.8°C) and hung from racks in the center of the radiation field for irradiation treatment. Most determinations were performed immediately, but samples for proximate analyses required storage. These samples were packaged *in vacuo* into high-barrier polyiminocaproyl/aluminum/polyethylene foil packages (Thayer et al., 1987) and held at −50°C until analyses were performed.

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Gamma-ray source

The gamma-ray source was a ^{137}Cs unit built by Lockheed Corporation, Marietta, Georgia, operating at a level of 0.108 kGy/min. Reference dosimeters from the National Physical Laboratory, Middlesex, United Kingdom, were used to calibrate the source. Dosimetry and dose distribution for the source have been described by Shieh et al. (1985). Samples were hung in bags from a stainless steel rack in the center of the gamma-ray field and exposed to doses of 0.234, 0.468, 0.937, 1.875, 2.812, 5.624, and 9.374 kGy. The temperature of the radiation chamber was maintained at 5 ± 0.5°C during irradiation by injecting the gas phase from liquid nitrogen. Sample temperature was monitored continuously during irradiation.

Thiamin

Thiamin was determined as previously described including blending of sample with 2% trichloroacetic acid (TCA), heating, centrifugation and determination by flow injection (FID) (Fox et al., 1992). Thiamin was oxidized to thiochrome by $K_3Fe(CN)_6$ and the concentration of the thiachrome was measured by its fluorescence, $\lambda_{excitation}$ = 365 nm, $\lambda_{emission}$ = 460 nm.

Riboflavin

Riboflavin was determined in the TCA extracts by FID from its fluorescence, $\lambda_{excitation}$ = 450 nm, $\lambda_{emission}$ = 530 nm.

Reducing capacity, sulfhydryl and pH

Total reducing capacities were determined by titration with dichlorophenolindophenol (DCPIP) (Fox et al., 1993). The sulfhydryl content was determined by use of Ellman's Reagent according to the method of Beverage et al., 1974. The pH was determined by placing a Ross (Orion Research, Inc., Boston, MA) combination pH electrode in contact with the meat.

Proximate analysis

The water and fat were determined by CEM methods (AOAC, 1990) with one modification. After determination of moisture content, the samples for fat determination were blended with 100 mL methylene chloride and slurries were quantitatively transferred to filter paper in a Buchner funnel and the methylene chloride removed by suction. Protein and ash were determined using CEM microwave furnaces: MAS-300 furnace for ash (CEM, 1989; Zhang and Dotson, 1994) and Kjel-FAST furnace for Kjeldahl digestion (CEM, 1987). After sulfuric acid/H_2O_2 digestion in the CEM oven, the alkalization and distillation of ammonia were carried

Table 1—Analysis of covariance of losses of thiamin in beef, lamb, pork and turkey with gamma irradiation as a covariate. General Linear Models Procedure, SAS. (Model: Ln [% thiamin] = Ln [% thiamin]$_0$ + slope × dose)

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	9	15.6	1.73	19.9	0.0001
Error	107	9.3	0.087		
Corrected total	116	24.9			

R-square	C.V.	Root MSE	LNT mean
0.626	6.90	0.295	4.29

Source	DF	Type I SS	Mean square	F value	Pr > F
D	1	13.9	13.90	159.76	0.0001
Species	4	0.756	0.189	2.17	0.077
D* Species	4	0.915	0.229	2.63	0.038

Parameter	Estimate	T for H0: parameter=0	Pr > |T|	Std. error of estimate
INTERCEPT	4.554 B	57.0	0.0001	0.80
Beef slope	−0.176	−7.7	0.0001	0.0229
Lamb slope	−0.083	−4.20	0.0001	0.0196
Pork slope	−0.104	−5.30	0.0001	0.0196
Tbre* slope	−0.119	−6.07	0.0001	0.0196
Tleg* slope	−0.103	−5.28	0.0001	0.0196

Contrast	DF	Contrast SS	Mean square	F value	Pr > F
Beef vs Pork	1	0.837	0.837	9.62	0.0025
Beef vs Lamb	1	0.493	0.493	5.67	0.019
Beef vs Tbre*	1	0.307	0.307	3.53	0.063
Beef vs Tleg*	1	0.500	0.500	5.75	0.018

* Tbre, turkey breast; Tleg, turkey leg.

out in a Kjeltec apparatus (AOAC, 1990). The ammonia was trapped in boric acid and titrated with 0.1N HCl to a bromcresol green/methyl red endpoint.

Mathematical analyses

Raw data were converted into regressions or subjected to analysis of covariance (ANCOVA) using appropriate procedures in the SAS software system (SAS Institute, Inc., 1987). Regressions were determined by the NLIN procedure and the ANCOVA by the GLM program. Regression coefficients were calculated from the following equation which expresses the dependence of the observed rate constant on the reductant concentration:

$$k_{observed} = k_o / (1 + k_r \times [R]) \quad (1)$$

RESULTS

Thiamin loss

Results of the ANCOVA of the log percent thiamin loss in the various species were compared (Table 1). To make the rate of loss data directly comparable considering variation in initial concentrations of thiamin, the data were converted to percent loss. Since the thiamin/hydroxyl radical reaction is first order with respect to thiamin, the percent loss figures were converted to log values to provide a linear plot. Note from those data in the column "Pr > F" the only significant factors were the dose (D) and intercept (initial values) due to various species (SPECIES). The effect of species on loss of thiamin (D*SPECIES) was significant. Upon comparison of the rate constants we found that this loss in beef was different from that in pork, lamb and turkey leg, but not turkey breast. None of the others was significantly different. The log [thiamin] regressions were initially calculated with dose × dose and dose × dose × species terms. Neither term was significant, that is, there was no curvature in the log plot, hence the terms were not included (Table 1).

Reductants

The reducing capacity of muscle tissues affects the rate of thiamin loss on gamma irradiation (Fox et al., 1993). In that study the range of values was wide enough to calculate the dependence of the rate of loss on reducing capacity. In our current study the reducing capacities of the tissues were clustered in a range of 16 to 20 mM reductant, which was not broad enough to calculate a significant slope. Irradiated samples were also titrated with DCPIP, but the effect of radiation dose on reducing capacity was not significant.

Table 2—Analysis of covariance of the losses of riboflavin in beef, lamb, pork and turkey with gamma irradiation as a covariate. General Linear Models Procedure, SAS. (Model: [riboflavin] = [riboflavin]$_0$ + slope × dose

Source	DF	Sum of squares	Mean square	F value	Pr > F
Model	14	1395.56	99.76	0.90	0.56
Error	104	11482.44	110.4		
Corrected total	118	12878.01			

R-square	C.V.	Root MSE	T mean
0.108368	10.71951	10.50753	98.0224971

Source	DF	Type I SS	Mean square	F value	Pr > F
D	1	625.02	625.0	5.66	0.019
Species	4	157.42	39.4	0.36	0.84
D* Species	4	233.61	58.4	0.53	0.72
D*D	1	107.14	107.1	0.97	0.33
D*D* Species	4	272.38	68.1	0.62	0.65

Parameter	Estimate	T for H0: parameter=0	Pr > |T|	Std. error of estimate
INTERCEPT	103.07 B	29.24	0.0001	3.53
Beef slope	−3.24	−1.28	0.204	2.54
Lamb slope	−3.95	−1.53	0.130	2.59
Pork slope	1.95	0.75	0.452	2.59
Tbre* slope	−2.17	−0.84	0.446	2.59
Tleg* slope	−1.98	−0.77	0.4455	2.59

* Tbre, turkey breast; Tleg, turkey leg.

Riboflavin

A net loss of riboflavin occurred in 9 of the 15 samples, but individually none of the rate constants was significant (Table 2). These data were subjected to an ANCOVA and collectively the loss was significant at the Pr > F level of 0.019. An analysis of the slopes indicated no difference in slopes due to species (Pr > | T | values all >0.05). We previously reported an increase in the amount of riboflavin measured in meats with a maximum at ca. 4 kGy (Fox et al., 1989), but we did not observe an increase in the current study, although there was no change up to about 3 kGy.

Sulfhydryl

One of the pork samples showed a decrease with radiation dose in sulfhydryl by Ellman's reagent, and one of the beef samples showed an increase. The remainder of the samples showed no significant changes. An ANCOVA showed no significant variation.

pH

Thiamin is stable to oxidation in acid solutions (Gubler, 1984) and a difference in pH could affect the rate of its loss. One pork sample had a low pH of 5.07 and one turkey leg had a high pH of 6.42. The average was 5.68 ± 0.28, within the range for post-rigor meats, but there no significant difference occurred in the pH of various species. Hence we could not verify whether pH had any effect on rate of thiamin loss.

Proximate analysis

Data for proximate analyses of the various species were compared (Table 3). The values for beef were all different from the highest or lowest values of the other species, primarily due to

Table 3—Proximate analyses of beef, lamb, pork and turkey leg and breast

Component	Component percent				
	Beef (rank)	Lamb	Pork	Tleg[c]	Tbre[c]
Protein	19.42[b] (low)	20.05[a,b]	20.73[a,b]	20.12[a,b]	23.45[a]
Fat	5.20[a] (high)	3.85[a,b]	3.08[a,b]	2.65[a,b]	1.38[b]
Moisture	70.81[b] (low)	72.69[a,b]	72.00[a,b]	74.57[a]	72.65[a,b]
Ash	0.84[b] (low)	0.96[a,b]	1.08[a]	0.97[a,b]	1.03[a,b]

[a,b] Means in the same row with no superscript in common are significantly different by ANCOVA.
[c] Tbre, turkey breast; Tleg, turkey leg.

Table 4—Comparison of thiamin losses from two separate studies

	Reducing capacity[c]		Chemiclearance[d]	
	[R]	kGy^{-1}	[R]	kGy^{-1}
Average	23.2[a]	0.051[b]	19.1[a]	0.100[b]
Standard deviation	7.7	0.025	4.5	0.048
Range	13.4–42.6	0.013–0.107	9.3–29.4	0.021–0.205

Regression[e]	Reducing capacity	Chemiclearance
k_0	0.557	0.153
k_r	0.462	0.0285
r	0.57 ($p < 0.0001$ @ 15 df)	N.S.

[a,b] Data with same superscripts are not significantly different from each other.
[c] Fox *et al.*, 1993.
[d] This study.
[e] For equation 1.

its higher fat content. Values for protein and water content were correspondingly lower. At the other extreme, turkey breast had a low fat content and a high protein content as expected for white muscle tissue. The extreme values in the ranges for each component were significantly different from each other, but neither extreme was significantly different from values in between. There was no correlation between proximate analysis values and losses of thiamin or riboflavin.

DISCUSSION

Vitamin loss

We compared our results with previously published values. Results for thiamin loss in beef, lamb, pork and turkey as related to the reducing capacity of the tissues (this study) were compared (Table 4) with a similar report of thiamin loss in beef, pork and chicken skeletal muscle and liver (Fox et al., 1993). Averages of the reducing capacity or the rates of thiamin losses were not significantly different, that is, the general ranges and scatter were the same. As noted previously (Fox et al., 1993), the reducing capacity is not the only factor controlling rates of thiamin loss, which probably explains the higher variation in rate constants as compared with reducing capacities. The regressions of the rate constants on the reducing capacities were calculated. The regression of the first set of data was significant but the regression for this study was not. The data in this study were clustered about 19–20 mM reducing capacities, and the range was too narrow to calculate a significant regression.

In a study of thiamin determination in matched chicken breasts (Fox et al., 1992) the coefficient of variation due to individual birds was ±15%, so that any variation beyond that range could be considered to be due to the treatment. In a study of loss of thiamin and other vitamins in pork chops and chicken breasts (Fox et al., 1989) loss of thiamin in pork was about three times that of chicken. However, that study was designed to determine vitamin loss in meats as processed and the chops and breasts were not under the same atmospheres or conditions. Ground meat was used for a study of vitamin loss as related to reducing capacity (Fox et al., 1993), whereas the meats were powdered in our current study, yet results were the same. Reducing capacities showed a 3-fold range in both studies (Table 4) and there was an 8-fold range of rate constants in the first study and a 10-fold range in our current study. Thus, while the rate of loss of thiamin in beef was twice that of lamb and was significant in view of an expected range of at least ten-fold, a two-fold variation is not particularly notable from a practical viewpoint.

Thiamin loss as a function of other tissue components

Of the several species, only beef was consistently different with a higher rate of thiamin loss. Beef also had higher fat and lower protein and moisture contents than the other meats. However, when beef was compared with the other meats, no correlation was found between thiamin loss and any of the tissue components, including water. In a study of the rehydration of freeze-dried pork (Fox et al., 1994), the rate of thiamin loss negatively correlated with water content, but the range of water contents was much greater. Lamb showed the lowest rate of thiamin loss, but none of the proximate analyses values differed between beef and lamb. As noted earlier, no correlation was found between thiamin loss and the reducing capacity of the several tissues we studied here. We concluded that the ranges of values were too low with respect to the range of thiamin loss values to establish significant relationships.

Vitamin loss in meats

Meats, in general, are not a major source of either B vitamin, since they contain about 1 μg thiamin/g and 2 μg riboflavin/g. Pork is an exception and contains ~ 10 μg thiamin/g (HNIS, 1987). Since first-order rate constants are independent of concentration (kGy^{-1}), the percent loss figures per given dose were directly comparable between all samples. At an average rate constant of 0.100 kGy^{-1}, the loss at 2 kGy would be 18%, but, based on the extremes of the ranges of the two studies reported, (0.0131 and 0.205 kGy^{-1}, Table 4), thiamin losses as low as 3% and as high as 34% could be expected. This amount of loss in all meats, except pork, would have little effect on the dietary intake of thiamin in the average consumer population. The major sources of thiamin and riboflavin, as well as the other B vitamins, are grain products, especially enriched flours and breads, in which the concentration of thiamin is about 8 μg/g and riboflavin 5 μg/g. From the data of Block et al. (1985) pork was calculated to supply about 9.00% of the thiamin in the American diet (Fox et al., 1989). At the average levels of thiamin losses, we found the loss in the American diet due to irradiation of pork to 3 kGy can be calculated as 2.3%, assuming that all pork and pork products were irradiated. The losses to the diet from irradiation of all other meats would be one-tenth of this value. Any detriment from such slight losses would seem to be more than compensated by the advantage of controlling bacteriological contamination through irradiation processing.

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